Can we withdraw anticoagulation in patients with antiphospholipid syndrome after seroconvertion?

CAN WE WITHDRAW ANTICOAGULATION IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME AFTER SEROCONVERTION?

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Abstract

The current mainstay of treatment in patients with thrombotic antiphospholipid syndrome (APS) is long-term anticoagulation, mainly with Vitamin K antagonist agents. Some recently available studies have created new ground for discussion about the possible discontinuation of anticoagulation therapy in patients with a history of thrombotic APS in whom antiphospholipid antibodies (aPL) are not detected any longer (i.e. aPL seroconversion).

We report the main points discussed at the last CORA Meeting regardingthe issue whether or notanticoagulation can be stopped after aPL seroconversion. In particular,we systematically reviewed the available evidence investigating the clinical outcome of APS patients with aPL seroconversion in whom anticoagulation was stopped when compared to those in whom therapy was continued regardless the aPL profile. Furthermore, the molecular basis for the aPL pathogenicity**,** the available evidence of non-criteria aPL and their association with thrombosis are addressed.

To date, available evidence is still limited to support the indication to stop oral anticoagulation therapy in patients with a previous diagnosis of thrombotic APS who subsequently developed a negative aPL profile. The identification of the whole risk profile for cardiovascular manifestations and possibly of a second level aPL testing in selected patients with aPL might support the eventual clinical decision but further investigation is warranted.

1. Introduction

High morbidity and mortality due to recurrence of thrombotic events is the main concern in patients with antiphospholipid syndrome (APS) [1]. Current evidence-based guidelines recommend long-term oral anticoagulation (OAC) as prophylaxis of new thrombotic manifestations of APS. The decision on the duration and intensity of this treatment should be based on the clinical features and immunological profile. According to the guidelines, patients with definite APS and a first venous thrombotic event should receive long-term OAC to an international normalized ratio (INR) target of 2.0–3.0 (the so called conventional-intensity anticoagulation). On the other side, patients with definite APS and an arterial thrombotic event should receive high-intensity anticoagulation therapy to an INR target between 3.0 and 4.0. However, long-term OAC has also been associated to a wide broad of hemorrhagic complications[2].

Especial attention has recently been payed to a small subset of patients who fulfill APS criteria but in whom aPL become persistently negative [3].

We systematically reviewed the available evidence investigating the clinical outcome of APS patients with aPL seroconversion (negativization) in whom anticoagulation was stopped when compared to those in whom therapy was continued regardless the aPL profile. Moreover, we aim to discussthe molecular basis for the aPL pathogenicityand the available evidence of non-criteria aPL and their association with thrombosis.

2. Systematic Review of the available evidence on aPL seroconversion.

The aim of this systematic review is to identify the available evidence on the clinical experience of thrombotic APS patients with persistent negative aPL. We also focused on those cases where anticoagulation was stopped after patients showed persistent negativity to conventional aPL tests.

2.1 Methods.

We performed a systematic review to identify and include in our study articles that reported clinical experience of thrombotic APS patients with persistent aPL seroconversion and patients that were

discontinued anticoagulation for that reason. Key words and subject terms used in the search included: ("antiphospholipid antibodies"[MeSH Terms] OR "aPL"[All Fields]) AND ("negativization"[All Fields]) AND ("anticoagulation"[All Fields] OR "treatment"[All Fields] AND "phospholipid"[All Fields]) OR ("antiphospholipid syndrome"[MeSH Terms] OR ("APS" [All Fields]).

The search strategy was applied to Ovid MEDLINE, In-Process and Other Non-Indexed Citation, EMBASE, Cochrane Central Register of Controlled Trials and Scopus from 1983 (year when the antiphospholipid syndrome was firstly described) to present; abstracts from EULAR and ACR were also screened. Inclusion criteria included either: a) follow up of thrombotic APS patients with persistent aPL seroconversion b) follow-up of thrombotic APS patients with persistent aPL seroconversion that were discontinued anticoagulation therapy. Studies that met the above-mentioned criteria were systematically analyzed by two independent reviewers (MR and IC). Disagreements were resolved by consensus; if consensus could not be achieved, a third party (SS) provided an assessment of eligibility. As the data on eligibility were dichotomous (eligible: yes/no), inter-rater agreement at both the title and abstract review and the full article review stages was determined by calculation of Cohen's kappa coefficient (k=0.85).

2.2 Results.

Out of 1006 screened studies, a total of four [3–6] met the inclusion criteria, including 47 thrombotic APS patients with persistently aPL seroconversion. Three out of four studies, including 23 thrombotic APS patients, described discontinuation of anticoagulation therapy after aPL seroconversion.

Characteristics of retrieved studies are summarized in Table 1.

2.2.1 Definition of aPL negativization.

The definition of aPL seroconversion/negativization was heterogeneous among studies. Two studies [4,5] included patients with 2 consecutive aPL seroconversion, however one of those studies [5] retested patients to confirm aPL disappearance after five years. Two studies [6,7] did not define the aPL negativization, however one study [7], reported patients with at least nine negative determinations of

criteria aPL testing. Of note, one study reported patients positive for aCL IgG at low titer in most cases and one patients positive for LA only but with negative results in a second determination [4]. Another study included mainly patients positive for LA only [5]; only one study [6] tested their cohort of patients for non-criteria aPL (anti-annexin A5 antibodies). Anti-beta 2 glycoprotein I (β2GPI) antibodies were not investigated in all the reported studies.

2.2.2 Follow up and Recurrences.

Medina and colleagues [5], investigated 24 primary APS patients with aPL seroconversion in a retrospective study, without stopping anticoagulation therapy. Since aPL disappearance and after 60 months of follow up, 11 out of 24 patients (45.8%) presented recurrence of thrombosis despite the continuation of the anticoagulant treatment. Among these 11 patients, 9 presented a DVT, one experienced an ischemic stroke and one patient developed pulmonary artery hypertension. 9 out of 24 patients (37.5%) had a past history of arterial thrombotic events (eight strokes and one mesenteric thrombosis) before aPL disappearance. This fact could place this group of patients at a higher thrombotic risk category.In addition, other non-thrombotic or non-criteria APS manifestations occurred in 6 patients, such as chronic skin ulcers in lower extremities in 4 patients and severe thrombocytopenia in two.Comarmond and colleagues[6] included 10 APS patients with prolonged disappearance of antiphospholipid antibodies (aPL) in whom anticoagulation therapy was discontinued. After a median duration of follow-up of 19 months (4-66.75) since the cessation of oral anticoagulantion, one out of 10 patients relapsed developing pulmonary embolism.The remaining two studies [4,7], included 6 primary APS and 7 primary APS patients, respectively. Anticoagulation therapy was discontinued in both studies and after a follow-up of 21 ± 12 months and 18 months, respectively, no recurrences were found.

The results of this systematic review seem to suggest that it is possible to discontinue antithrombotic treatment in some patients with primary APS in whom aPL became persistently negative. However, altogether these reports do not allow to get a clear conclusion about the withdrawal of VKA treatment based on the analysis of the literature.

3. Molecular basis for the antiphospholipid antibody pathogenicity.

The underlying mechanisms by which aPL can induce a thrombophilic phenotype and clinical manifestations are still under investigation. In this section, we will review the available evidence on molecular basis for aPL pathogenicity based mainly on the studies carried out by a group of the authors of the present manuscript (SS, RM, BML, LPC, CMJ). The aim to review these mechanisms is to show the profound changes that aPL induces at different levels of coagulation and inflammatory pathways.

3.1 Tissue factor and endothelial dysfunction.

Pro-coagulant cell activation, accompanied with tissue factor (TF) expression, and TF pathway up regulation are key events explaining the pathophysiology of thrombosis in patients with APS [8]. In addition, it has been shown that TF signalling activities in APS, induced by aPL, are mainly mediated by protease-activated receptors (PARs). Accordingly, PAR1- and PAR2-induced signalling is directly involved in the constitutive mitogen-activated protein kinase (MAPK) activation [9] and the increased expression of the pro-inflammatory cytokine vascular endothelial growth factor (VEGF) and its receptor Flt1 [10]. Similar results have been reported in endothelial cells (ECs),platelets, and monocytic cell lines and in in vivo models of aPL-induced thrombogenicity [11–13].

aPL are also responsible for the altered protein profile of monocytes related to thrombosis development, including deregulated expression of annexin A1 (AnxA1), annexin A2 (AnxA2), ubiquitin Nedd8, Rho A protein, PDI and Hsp60. These proteins have been shown to be associated with the induction of a pro-coagulant state, as well as autoimmune-related responses [14].

Early endothelial dysfunction [15] and increased carotid intima media thickness (CIMT)have been also observed in APS [16]. In a recent study, including a cohort of 43 APS patients, higher aPL-IgG titers showed a strong association with the development of thrombotic events and carotid intima-media thickness increase[17].

3.2 Oxidative Stress.

Several studies have evidenced that oxidative stress is directly involved in the pathophysiology of some APS features. In the setting of APS, aCL antibodies seem to play an important role in the oxidative status by inducing nitric oxide (NO) and superoxide production, resulting in enhanced levels of plasma peroxynitrite, a powerful pro-oxidant substance [18]. aCL antibodies levels positively correlated with plasma levels of F2-isoprostanes, sensitive markers of in vivo lipid peroxidation, indicating enhanced oxidative stress in APS [19,20].

Perez-Sanchez et al showed [21] an increased production of reactive oxygen species (ROS) by monocytes and neutrophils in APS that disturbs the redox status and in turn may influence the expression of prothrombotic and proinflammatory molecules.

3.3 Toll like receptors.

Different studies have demonstrated that up-regulation of Toll-like receptors (TLR) is playing a role in APS pathogenesis. Both TLR4 on endothelial cells and TLR 7 & 8 in plasmacytoid dendritic cells and monocytes respectively, can be modulated by aPL eventually inducing biological responses in the cells [22, 23].

3.4 NETosis.

Apart from the generation of ROS and the release of microbicidal molecules from granules, antimicrobial activity called neutrophil extracellular traps (NETs) activation and release (or NETosis), has recently been described and can promote tissue damage, thrombosis, atherosclerosis and autoimmunity. NET generation has been recognized as an important activator of the coagulation cascade, being deeply associated with deep vein thrombosis 24]. NETs can regulate thrombosis through several mechanisms, accordingly NETosis has been suggested to represent an additional mechanism of thrombosis mediated by aPL. Knight and co-workers recently showed that NETs circulate at high levels in the plasma of APS patients, even between thrombotic episodes [25]. Indeed, freshly isolated neutrophils from APS patients are primed to undergo spontaneous NETosis when cultured ex vivo. Mechanistically, anti-β2GPI IgG promotes NETosis by engaging β2GPI protein on the neutrophil surface; this process is independent of the Fc receptor, but does require

ROS production and TLR4 signalling. Further, and pointing to disease relevance, anti-β2GPI-stimulated NETs promote thrombin generation in vitro and in vivo [26].

3.5 Genomic and epigenetic.

aPL are also able to induce genomic and epigenetic modulation, creating a pro-thrombotic state. A recent study by Perez-Sanchez et al.described specific gene profiles in monocytes from APS patients, explaining the pro-atherothrombotic alterations (mainly related to aPL titres, and promoted in vitro by aPL) [27].

Current genome-wide studies have further shown that the human genome is pervasively transcribed and produces many thousands of regulatory non-protein-coding RNAs (ncRNAs), including microRNAs, small interfering RNAs, and various classes of long ncRNAs. It is now clear that these RNAs fulfil critical roles as transcriptional and post-transcriptional regulators and as guides of chromatin-modifying complexes. Among them, miRNAs are small ncRNAs ubiquitously expressed, have a profound influence in the immune system and a relevant role in the pathogenesis of autoimmune disorders.Preliminary studies have shown that several members of the miR 17-92 cluster (miR-20a and miR-19b) regulate the expression of TF in monocytes from patients with APS and systemic lupus erythematosus (SLE) [28]. A reduction in their expression has been found to significantly correlate with the over-activation of TF. The reduction in the expression of these miRNAs could therefore contribute to the prothrombotic state characteristic of APS and SLE patients [28].

The relevance of these mechanisms, when considering stop anticoagulation, relies on the following open question: are these profound changes in thrombotic, inflammatory and oxidative status reversible when aPL become negative? Moreover, if this is the case, how long after negativitation of aPL all these changes are back regulated?

4. Non-classification criteria antiphospholipid antibodies and thrombosis.

Data gathered in recent years explored the relationship between three groups of antiphospholipid antibodies, not currently included in the classification criteria and the development of thrombotic events.

In this section, we will summarize available data on Prothrombin/phosphatidylserine complex, antibodies directed to the Domain I of β2GPI and IgA aPL, and their association with thrombotic risk.

4.1. Prothrombin/phosphatidylserine complex.

Early reports suggest an association between APS and antibodies to prothrombin, directed either to prothrombin in solid phase (aPT) or to a complex of phosphatidylserine/prothrombin (aPS/PT). While these antibodies are known to be directed to the same molecule, they differ in their immunological characteristics and clinical associations [29]. Emerging evidence supports the clinical utility of aPS/PT over that of aPT in the setting of APS [30]. A recent systematic review of the topic analyzed available data on more than 7000 patients and controls from 38 studies on aPT and 10 studies on aPS/PT. aPS/PT was associated with both arterial and/or venous thrombosis and this association was stronger for aPS/PT when compared to aPT (OR 5.11 [95%CI 4.2-6.3] vs. 1.82 [95%CI 1.44-2.75], respectively) [31].Data coming from a study in 265 Japanese patients with systemic autoimmune diseases showed that aPS/PT conferred a 3.6 fold increase risk for APS [32]. Several studies that followed confirmed the association between aPS/PT and the clinical manifestations of APS [33,34]. A large study in a cohort of 728 patients suspected of having APS, in the absence of aCL or anti-β2GPI showed that 41 of them (5.6%) had elevated levels of aPS/PT with thrombotic events occurring in 50% of these cases [35]. This finding strongly suggests that aPS/PT may represent a valuable diagnostic test in a small group of patients with APS like manifestations even in the absence of classification laboratory criteria.

When evaluating the diagnostic accuracy of several aPL specificitiescombinations, the profile including LA + anti-β2GPI + aPS/PT held the best diagnostic accuracy for APS even when subdividing the study group into those with thrombosis and those with pregnancy loss[36]. In addition, aPS/PT have been confirmed as a strong risk factors for thrombosis, irrespective of the site and type of thrombosis. Their presence incurred in an odds ratio (OR) for thrombosis 3 to 18 times higher than in various control groups [37].

While a close association has been reported between aPS/PT and the LA, our data showed that aPS/PT and LA are not interchangeable tests but independent, and therefore, additive risk factors for thrombosis [38].

4.2 Antibodies directed to the Domain I of β2GPI.

Anti-β2GPI antibodies have been reported to target each of the 5 different domains of the β2GPI molecule [39]. However, it is the N-terminal domain, designated domain I (or DI), that has been described of importance in the pathogenesis of APS [40,41]. De Laat et al reported that positivity for anti-DI is associated with an increased risk of thrombosis [42]. A subsequent multicenter study by the same group [43] reported a 3.5-fold increase in the risk of developing vascular thrombosis in patients positive for IgG anti-DI when compared with those without this antibody. In addition, the risk of having pregnancy morbidity in this population also displayed a 2.4-fold increase. High-risk APS patients, those with triple positivity for aPL, including aCL, anti-β2GPI and the LA, have been reported to also have higher titers of circulating anti-DI when compared with those with double or single aPL positivity [44]. A recent study from Pericleous et al has reported that not only positivity for IgG anti-DI but also for the IgM and IgA isotypes of anti-DI are strongly associated with APS [45]. In addition, using the novel β2GPI-DI chemiluminescence assay (CLIA), Mahler et al showed an OR for thrombosis of 9.5 in a cohort of 106 patients with APS and high levels of anti-DI [46].

Anti-DI antibodies are of increased clinical interest, indeed. However, it is now clear that not all anti-β2GPI target DI, and some anti-β2GPI react with other domains of the β2GPI molecule. Antibodies to DIV/V have been reported in asymptomatic patients, leprosy, and in children with atopic dermatitis [47, 48].In this respect, anti-DIV/V antibodies were reported among asymptomatic aPL carriers, those with no evidence of aPL-related clinical manifestations or any underlying autoimmune disease. In this study, the authors suggested that the ratio between antibodies to DI versus DIV/V might be predictive for systemic autoimmunity [49].

While anti-D1 antibodies seem to be higher predictive for thrombotic manifestations than the test against the native molecule, anti-D1 assay displays a lower sensitivity suggesting that anti-D1 cannot substitute the standard test in the routine [49]. There are actually anecdotal reports of anti-D1 positivity with negativity

for anti-β2GPI molecule (and for other aPL), but the values were in the borderline area and further studies are necessary to investigate such an aspect [50].

4.3 IgA aPL

Controversy still exist as to whether patients with features of APS, negative for other IgG or IgM aPL, may benefit from IgA aPL testing and, if they are tested and found positive, their clinical relevance [51]. In general, IgA aPL are found in association with other isotypes (i.e., IgG and/or IgM), and mainly associate to skin ulcers, Raynaud's phenomenon, livedo, or cutaneous vasculitis [52]. In addition, these antibodies have been reported as the dominant isotype in Afro-Caribbeans [53] and in Afro-Americans [54], usually present transiently or at low or moderate titers and in the absence of any aPL related clinical manifestation.

Interestingly, recent data from the transplantation field suggest that pretransplant IgA aPL, specifically IgA anti-β2GPI, may serve as an independent risk factor for early graft thrombosis after renal transplantation [55], attributing a potential new role for IgA aPL as a biomarker for early transplant failure.

4.4 Anti-annexin A5 and antivimentin antibodies

Other antibody specificities, including anti-annexin A5 and antivimentin antibodies, might be eligible for thrombotic risk assessment just in selected patients, particularly when other aPL tests are negative with the presence of clinical APS signs and/or symptoms. Indeed, further investigations are needed to assess their role in the diagnostic algorithm for APS [56].

Of note, very recently, Conti et al. [57] described a patient with catastrophic APS, the most severe variant of the disease, who tested negative to the conventional aPL but positive for aPL in thin layer chromatography immunostaining and vimentin/cardiolipin antibodies by ELISA test, highlighting how relevant non-criteria antibodies, in selected cases, can potentially be. However, anti-vimentin antibodies have been found in a wide panel of systemic autoimmune diseases in addition to APS, raising the question of their specificity [58].

To date, laboratory criteria for APS include the assays test for the presence of LA, aCL and anti-β2GPI antibodies, however, in patients with persistent disappearance of aPL, some authors suggest a second level screening of non-criteria aPL before stopping the anticoagulant treatment.

5. Conclusions

aPL seroconversion and its definition is a rising topic of interest in the field of APS. Although there are reports in the literature supporting the discontinuation of antithrombotic treatment in some patients with primary APS in whom aPL became persistently negative, we still do not have enough data to make sound recommendation whether or not and when stopping oral anticoagulation therapy in APS patients with seroconversion.

In particular, there is concern about the changes induced by aPL in pro-thrombotic and pro-inflammatory status and whether or not such biological changes can disappear at the same time of the seroconversion.

The disappearance of formal classification aPL tests should be confirmed over time and second-level diagnostic test should be also investigated. In addition, the whole cardiovascular risk profile should be also evaluated.

Ideally, OAC thromboprophylaxis should be withdrawn when the risks of this therapy outweigh the risks of thrombotic recurrence. In this sense, the following definition of low-risk profile for thrombotic recurrence after OAC discontinuation could be: *no more than a single prior non-critical first venous thrombotic event in the presence of a known transient precipitating risk factor (smoking, hypertension, recent surgery, etc.) together with a low-risk aPL profile (single aPL that becomes persistently negative over a long period of time; i.e., more than 2 years).*

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Legend of Tables:

Table 1.The table reports the main characteristics of the four studies that met the inclusion criteria on aPL seroconversion.

Study, year	Criado-García et al. 2008	Medina et al. 2017	Comarmond et al. 2017	Bazàn et al. 2017
Study's design	Prospective	Retrospective	Retrospective	Retrospective
Patients	6 Primary APS	24 Primary APS	10 APS	7 Primary APS
Previous LA positivity (%)	16,70%	N/A	N/A	86%
Previous aCL (IgG/IgM) positivity (%)	100%	87,50%	N/A	28,60%
Previous anti-β2GPI (IgG/IgM) positivity (%)	0%	N/A	N/A	0%
Non Criteria aPL tested	N/A	anti-annexin A5 antibodies	N/A	N/A
Time of aPL positivity (months)	10-53	109,4 ± 80,7	N/A	At least two aPL determinations, performed 12 weeks apart
Mean time of anticoagulation therapy (months)	25 ± 18	N/A	21 (9–118)	111 ± 52,4
Definition of aPL negativization	2 consecutive negative determinations	≥2 subsequent negative aPL determinations, retested after 5 years	N/A	At least nine negative determinations
Follow up after anticoagulation discontinuation (months)	21 ± 12	60	19 (4–66.75)	18
Recurrences at follow-up	None	45.8%: DVT in 37,5%, ischemic stroke in 4,2%, pulmonary artery hypertension in 4,2%. Other non-thrombotic APS manifestations were chronic ulcers in lower extremities and severe thrombocytopenia	One out of 10 patients (10%) relapsed developing PE	None

Table 1.

APS (Antiphospholipid Syndrome); aPL (antiphospholipid antibodies): LA (Lupus Anticoagulant); aCL (anticardiolipin); β2GPI (and anti-β2 glycoprotein-I) ; DVT (Deep Vein Thrombosis); PE (Pulmonary Embolism)